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JC07 Rec'd PCT/PTO 08 MAR 2007

PCT Applicant's Guide - Volume II - National Chapter - US

Annex US.II, page 1

Express Mail No. el651822109us

FORM PTO-1390 (REV 10-95)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				15675p393
				U.S. APPLICATION NO. (If known, see 37 CFR 1.5) <b>10/070929</b>
INTERNATIONAL APPLICATION NO. PCT/FR00/02506	INTERNATIONAL FILING DATE September 12, 2000	PRIORITY DATE CLAIMED		
TITLE OF INVENTION SET FOR HEAT TREATMENT OF BIOLOGICAL TISSUES AND METHOD USING SAME				
APPLICANT(S) FOR DO/EO/US Rares-Vasile Salomir; Jacobus Adrianus de Zwart; Frederic Vimeux; Christ Moonen				
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:				
<ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b)) and PCT articles 22 and 39(1).</li> <li>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> has been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</li> <li>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)). <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> have been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</li> <li>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol>				
Items 11. to 16. below concern document(s) or information included:				
<ol style="list-style-type: none"> <li>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>13. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</li> <li>14. <input type="checkbox"/> A subsequent specification.</li> <li>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>16. <input checked="" type="checkbox"/> Other items or information: Request for priority; Transmittal of formal drawings t/w 9 sheets, 10 figures; Preliminary exam report; English translation of the report; forms pct/ib 301&amp;301; request</li> </ol>				

JC10 Rec'd PCT/PTO 08 MAR 2002

US

Annex US.II, page 2 PCT Applicant's Guide - Volume II - National Chapter - US

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)		INTERNATIONAL APPLICATION NO.		ATTORNEY'S DOCKET NUMBER	
10/070929		PCT/FR00/02506		15675p393	

<p>17. <input checked="" type="checkbox"/> The following fees are submitted:</p> <p><b>BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5) ):</b></p> <p>Neither international preliminary examination fee (37 CFR 1.482 nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by EPO or JPO . . . . . <b>\$1040.00</b></p> <p>International preliminary examination fee (37CFR1.482)not paid to USPTO but International Search Report prepared by the EPO or JPO. . . . <b>\$890.00</b></p> <p>International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee paid to USPTO (37 CFR 1.445(a)(2)) . . . . . <b>\$740.00</b></p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) . . . . <b>\$710.00</b></p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) . . . . . <b>\$100.00</b></p> <p style="text-align: right;"><b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b></p> <p>Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 15%;">CLAIMS</th> <th style="width: 15%;">NUMBER FILED</th> <th style="width: 15%;">NUMBER EXTRA</th> <th style="width: 15%;">RATE</th> <th style="width: 15%;"></th> <th style="width: 15%;"></th> </tr> <tr> <td>Total claims</td> <td>6 - 20 =</td> <td>0</td> <td>X \$18.00</td> <td>\$</td> <td>0.00</td> </tr> <tr> <td>Independent claims</td> <td>1 - 3 =</td> <td>0</td> <td>X \$84.00</td> <td>\$</td> <td>0.00</td> </tr> <tr> <td colspan="4">MULTIPLE DEPENDENT CLAIM(S) (if applicable)</td> <td>+</td> <td>\$280.00</td> </tr> <tr> <td colspan="4" style="text-align: right;"><b>TOTAL OF ABOVE CALCULATIONS =</b></td> <td>\$</td> <td>1170.00</td> </tr> </table> <p>Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).</p> <p style="text-align: right;"><b>SUBTOTAL =</b></p> <p>Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).</p> <p style="text-align: right;"><b>TOTAL NATIONAL FEE =</b></p> <p>Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property +</p> <p style="text-align: right;"><b>TOTAL FEES ENCLOSED =</b></p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 60%;"></td> <td style="width: 20%; text-align: center;">\$ Amount to be: refunded</td> <td style="width: 20%; text-align: center;">\$</td> </tr> <tr> <td></td> <td style="text-align: center;">charged</td> <td style="text-align: center;">\$</td> </tr> </table>	CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE			Total claims	6 - 20 =	0	X \$18.00	\$	0.00	Independent claims	1 - 3 =	0	X \$84.00	\$	0.00	MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+	\$280.00	<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$	1170.00		\$ Amount to be: refunded	\$		charged	\$	<p><b>CALCULATIONS FOR PTO USE ONLY</b></p>
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	charged	\$																																			

a. ☒ A check in the amount of \$ 1170.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_ to cover the above fees.  
A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 022666. A duplicate copy of this sheet is enclosed.

**NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

Blakely, Sokoloff, Taylor & Zafman LLP  
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\_\_\_\_\_  
SIGNATURE

Eric S. Hyman  
NAME

30,139  
REGISTRATION NUMBER

Attorney Docket No.: 15675p393

10070929 062602  
**107070929**

**JC10 Rec'd PCT/PTO 08 MAR 2002**  
Express Mail No. cl651822109us

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

RARES-VASILE SALOMIR, ET AL.

Application No.:

Filed:

For: **SET FOR HEAT TREATMENT OF  
BIOLOGICAL TISSUES AND METHOD  
USING SAME**

Art Group:

Examiner:

Assistant Commissioner for Patents  
Washington, D.C. 20231

**TRANSMITTAL OF FORMAL DRAWINGS**

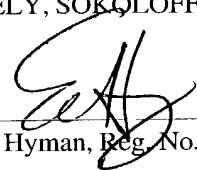
Sir:

Enclosed herewith for filing in the above-identified U.S. Patent Application are the formal drawings, 9 sheets including 10 Figures. Applicant hereby authorizes any additional extension or petition fees under 37 C.F.R. §1.17 or credit for any overpayment to our Deposit Account No. 02-2666. A copy of the Fee Transmittal sheet is enclosed.

Respectfully submitted,

BLAKELY, SOKOLOFF, TAYLOR & ZAFMAN

Dated: 3/8/02

  
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10/070929

JC10 Rec'd PCT/PTO 08 MAR 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. :

U.S. National Serial No. :

Filed :

PCT International Application No. : PCT/FR00/02506

VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

My name and post office address are as stated below;

That I am knowledgeable in the French language in which the below identified international application was filed, and that, to the best of my knowledge and belief, the English translation of the international application No. PCT/FR00/02506 is a true and complete translation of the above identified international application as filed.

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application issued thereon.

Date: February 25, 2002

*Caroline Bentley*

Full name of the translator :

Caroline Ann BENTLEY

For and on behalf of RWS Group plc

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**10/070929**

**JC10 Rec'd PCT/PTO 08 MAR 2002**

Our Ref. No.: 15675.P393  
Express Mail No. EL651822109US

UTILITY APPLICATION FOR UNITED STATES PATENT

FOR

**SET FOR HEAT TREATMENT OF BIOLOGICAL TISSUES AND METHOD USING  
SAME**

Inventor(s):

Rares-Vasile Salomir  
Jacobus Adrianus de Zwart  
Frederic Vimeux  
Christ Moonen

WO 01/19457

9/PRTS 1 -

PCT/FR00/02506

SET FOR HEAT TREATMENT OF BIOLOGICAL TISSUES AND METHOD  
USING SAME

5 The invention relates to the field of apparatuses  
intended for local hyperthermia therapies. The  
invention also relates to a method of using apparatus  
of this sort.

10 Local hyperthermia therapies consist in heating,  
locally, a target zone of biological tissue. When this  
type of therapy is used in the context of gene therapy,  
the heat may, for example, be used for its action on a  
heat-sensitive promoter. Heat may also be used to  
necrose biological tissue and to ablate tumors.

15 Also, local hyperthermia therapies offer numerous  
advantages. These advantages are both qualitative and  
economic. From the qualitative point of view, they  
offer, for example, strong potential for the control of  
20 treatments such as gene therapies, the local  
application of medications, the ablation of tumors,  
etc. From the economical point of view, they are  
compatible with the ambulatory treatment of patients,  
they make it possible to reduce the length of time  
25 spent in hospital, etc.

In hyperthermia therapies, the heat may, for  
example, be provided by a laser, microwaves or radio-  
frequency waves, focused ultrasound, etc. In general,  
30 local hyperthermia therapies allow medical operations  
where the invasive nature is reduced to the minimum.  
However, among the aforementioned energy types, focused  
ultrasound is particularly beneficial since it makes it  
possible to heat the focusing zone, in a noninvasive  
35 way, deep within a biological body, without  
significantly heating the tissue in the vicinity of the  
focusing zone.

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In all cases, the temperature of the target zone and of its immediate surroundings, during the treatment, must be accurately and continuously controlled, although the supply of energy is localized and fast (of the order of a few seconds). To this end, it is possible to fit temperature probes in the target zone and its immediate surroundings. However, it is also possible to use Magnetic Resonance Imaging (MRI). This is because MRI makes it possible to obtain an accurate map of the temperature distributions and detailed anatomical information. Furthermore, MRI allows noninvasive control of the temperature.

Devices for controlling the temperature during treatments by focused ultrasound are already known, based on magnetic resonance thermometry. Devices of this sort are in particular described in the following documents: "Control system for an MRI compatible intracavitary ultrasounds array for thermal treatment of prostate disease", Smith NB et al., Proceedings of the annual meeting of the International Society of Magnetic Resonance in Medicine, 1999, p. 672 and "Real time control of focused ultrasound heating based on rapid MR thermometry", Vimeaux FC et al., *Invest. Radiol.* 1999, 34, p. 190-193. In the devices described in these documents, the retrocontrol of the heat provided by the focused ultrasound, by virtue of the maps obtained by MRI, is of the PID (Proportional Integral and Derivative) type. Furthermore, with these devices, control of the heat supplied to the tissue is based on taking into account a temperature measured in the focusing zone of the ultrasound equipment, or corresponding to a mean obtained from the spatial temperature distribution in the mapped zone.

Figure 1 shows the temporal change in the mean temperature of the focusing zone, processed by virtue of the device described in the first of these

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documents. In this figure, the temperature increases up to a plateau corresponding to the temperature that it is desired to reach in the focusing zone. It may be noted that the temperature desired in the focusing zone is reached only after a period of about 30 minutes.

Figure 2 shows the temporal change in the mean temperature in the focusing zone, processed by virtue of the device described in the second of the documents mentioned above. It may be noticed that the temperature desired in the focusing zone is reached in less than 2 minutes. However, variations in the desired temperature, of plus or minus 4°C, are observed.

One aim of the invention is to provide equipment for the heat treatment of a target zone of biological tissue, enabling the temperature desired in the target zone to be obtained quickly and at the same time the temperature in this target zone to be maintained and controlled with increased accuracy, compared to that which was possible with the techniques of the prior art.

This aim is achieved, according to the invention, by virtue of equipment for the heat treatment of a target zone of biological tissue, comprising:

- energy generating means for supplying energy locally in the target zone;
  - means for measuring and recording the temperature in the target zone;
  - a control unit comprising means for determining, from the temperature measured in the target zone, the amount of energy having to be supplied to the target zone, and means for controlling the energy generating means to deliver this power value;
- characterized in that the control unit furthermore comprises means of numerically processing, point by point, the spatial temperature distribution in the



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target zone and its surroundings, in order to calculate temperature gradients.

5 The heat treatment equipment according to the present invention takes into account the actual spatial temperature distribution in the target zone, but also in the surroundings of this zone. That is to say that it takes into account and processes, point by point, this spatial distribution. Unlike the heat treatment  
10 equipment of the prior art, the spatial temperature distribution is used to deduce therefrom temperature gradients and not merely averages. This makes it possible to estimate, with increased accuracy, the amount of energy which must be applied and therefore to  
15 achieve the desired temperature more quickly and to maintain the temperature of the biological tissue with greater stability.

Advantageously, the control unit of the heat  
20 treatment equipment according to the invention furthermore comprises means for estimating the local heat energy losses, from an estimate of the heat conduction and of the spatial temperature distribution in the target zone and its surroundings. This is  
25 because the information supplied by the value of the temperature gradients and the taking into account of an estimate of the local heat losses not only make it possible to understand the way in which the treated biological tissue has reacted to the heat already  
30 applied thereto, but furthermore, make it possible, by virtue of the prediction concerning the way in which the biological tissue will react to the heat. This also makes it possible to make the temperature of the heat-treated tissue change more quickly toward the desired  
35 temperature and to maintain the temperature of the biological tissue with greater stability.

Advantageously, the energy generating means of the heat treatment equipment according to the invention emit focused ultrasound. This is because focused ultrasound makes it possible to supply heat to a very localized zone, in a noninvasive manner, even if this zone is located deep within a human body or an animal. Furthermore, the focusing makes it possible for the tissue near to the zone of treated biological tissue not to be significantly heated.

10

Advantageously, the means for measuring and recording the spatial temperature distribution of the heat treatment equipment according to the invention comprise a magnetic resonance imaging apparatus. This is because MRI allows measurement of the temperature, which is noninvasive, accurate and well resolved in many points of the mapped zone. The data collected by MRI are, furthermore, easily processed numerically.

20

Advantageously, the heat treatment equipment according to the invention comprises means for evaluating the spatial distribution, in the target zone and its surroundings, of the energy supplied to the target zone.

25

According to another aspect, the invention is a method for regulating equipment for heat treating a target zone of biological tissue, comprising the step consisting in locally applying energy to the target zone,

30

characterized in that it further comprises the steps consisting in

- evaluating the temperature gradients in the target zone and its surroundings; and
- thereby deducing the energy to be applied to the target zone in order to reach the desired temperature.

35

Advantageously, this method furthermore then comprises the step consisting in estimating the local energy losses, in the target zone and its surroundings.

5 Advantageously, this method furthermore comprises the step consisting in evaluating the spatial distribution, in the target zone and its surroundings, of the energy supplied to the target zone.

10 Other aspects, aims and advantages of the invention will become more clearly apparent on reading the following detailed description.

The invention will also be better understood by means of the appended drawings in which:

- 15 - figure 1 shows the temporal change in temperature of a target zone, when the target zone is treated using heat treatment equipment of the prior art;
- figure 2 shows the temporal change in temperature of a target zone treated by another heat treatment
- 20 equipment of the prior art;
- figure 3 shows schematically the heat treatment equipment according to the invention;
- figure 4 is a flow diagram of the regulation method according to the present invention;
- 25 - figure 5 shows the temporal change in the Laplacian type, during heat treatment by the equipment according to the present invention, corresponding to an *in vitro* experiment described below;
- figure 6 shows the results of a series of experiments
- 30 aiming to examine the effect of estimation errors on the energy losses, represented by the heat diffusivity and the absorption coefficient of the ultrasonic energy;
- figure 7 shows the temporal change in the maximum
- 35 temperature, measured during the thermal treatment of biological tissue, by the equipment according to the present invention, corresponding to the same *in vitro* experiment as that of figure 5;

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- figure 8 shows the temporal change in the amplitude of the signal emitted by the generator of the equipment according to the present invention, during the thermal treatment of a biological tissue, corresponding to the same *in vitro* experiment as that in figures 5 and 7;
- figure 9 shows the temporal change in the maximum temperature, during the heat treatment, of a biological tissue, by the equipment according to the present invention, corresponding to another *in vitro* experiment, with three temperature stages, and described below; and
- figure 10 shows the temporal change in the maximum temperature, measured during the heat treatment by the equipment according to the present invention, corresponding to an *in vivo* experiment described below.

One of the embodiments of the invention is described below in detail. By way of example, this embodiment of the invention corresponds to equipment for local hyperthermia treatment by focused ultrasound, controlled by MRI.

As shown in figure 3, equipment of this sort comprises:

- energy generating means 100;
- mapping means 200;
- a control unit 300; and
- a sample holder 400 for the biological tissue 410 to be treated.

In the embodiment of the invention described here, the energy generating means 100 consist of a transducer 110, a sinusoidal signal generator 120, an amplifier 130 and a converter 140, connecting the sinusoidal signal generator 120 to the control unit 300.

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The transducer 110 operates at 1.45 MHz. A transducer 110 of this type is, for example, marketed by Speciality Engineering Associates® (Soquel, California). Its diameter and its focal length are  
5 38 mm and 25 mm, respectively.

The sinusoidal signal generator 120 is, for example, of the FG110 type, marketed by Yokogawa® (Tokyo, Japan).  
10

The amplifier 130 is, for example, of the KMP 170F type, marketed by Kalmus® (Bothell, Washington). This amplifier 130 has a power gain of 58 dB.

The converter 140 is, for example, a series IEEE488 converter, marketed by I. O. Tech.® (Cleveland, Ohio).  
15

The mapping means 200 make it possible to measure and record the spatial temperature distribution. They  
20 comprise, for example, an MRI apparatus of the Bruker Biospec type marketed by Bruker® (Ettlingen, Germany). This apparatus uses a 4.7 T magnet which is equipped with a 120 mm diameter insert, which generates magnetic  
25 field gradients (the maximum value of the gradient is 0.193 T/m).

The control unit 300 comprises, in particular, a work station 310 of the Alpha PW 500a MHz type,  
30 marketed by Digital®.

The control unit 300 also comprises means for evaluating and numerically processing the spatial temperature distribution 320, means for determining the  
35 power value 330 having to be supplied to the target zone, means for estimating local losses of heat energy 340 and means 350 for controlling energy generating means 100. The control means 350 instruct the energy

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generating means 100 to deliver the power value supplied by the means 330 for determining the power value.

5           The sample holder 400 comprises a rat support 420, made of plexiglas<sup>®</sup>. This support 420 contains the transducer 110 and a surface coil (not shown in figure 3). A sample holder 400 of this type has already been described in the documents "*Fast lipid suppressed MR*  
10 *temperature mapping with echo-shifted gradient echo imaging and spectral-spatial excitation*", by Zwart JA et al., 1999, Magn. Res. Med., 42, p. 53-59; and "*On the feasibility of MRI-guided focused ultrasound for local induction of gene expression*", Madio DP et al., 1998,  
15 J. Magn. Res. Imaging. I, 8, p. 101-104. The support 420 is placed in the plexiglas<sup>®</sup> tube which is partly filled with water. The transducer 110 is positioned so that the focusing point 460 of the ultrasound is located approximately 10 mm deep within the biological  
20 tissue 410. During the *in vitro* measurements, a temperature probe 430 is inserted into the biological tissue 410 consisting of a piece of fresh meat, so as to have a temperature reference. This probe 430 is, for example, a thermocouple of the Digi-Sence DualLog type,  
25 marketed by Cole-Parmer Instrument Co.<sup>®</sup> (Vernon Hill, Illinois).

          The preparation of the samples for the *in vitro* and *in vivo* experiments is carried out as follows. Male  
30 rats of the Wistar race from 325 to 500 g are taken. The latter are anesthetized by combining 1% by volume of halothane with a mixture consisting of 7 volumes of nitrous oxide (N<sub>2</sub>O) to 3 volumes of oxygen, according to an approved protocol. In order to improve the  
35 penetration of the ultrasound beam into the biological tissue 410, the thigh of the rat (which is between the transducer and focal point) is depilated, using a product provided to this end and known to a person

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skilled in the art. During the *in vivo* measurements, the endorectal temperature of the rats is recorded. The body temperature of the rats is maintained at 35°C by immersion of the bodies of the rats in a bath, the temperature of which is regulated for this purpose. After the heat treatment, the rats are sacrificed.

The mapping means 200 are used by implementing an Echo gradient sequence, with the following parameters: TR = 50 ms, TE = 15 ms, matrix size = 64 x 63, three k lines per TR, FOV = 64 mm x 63 mm, where TR is the repetition time, TE is the echo time and FOV is the field of view. The data are obtained by MRI, from a section 2 mm in thickness, perpendicular to the ultrasound transducer and comprising the focal point of the focused ultrasound. The temporal resolution of the maps obtained by MRI is 1.05 s. The spatial resolution of the maps obtained by MRI is 1 x 1 x 2 mm<sup>3</sup>. The maps of the temperature measured by magnetic resonance are obtained from measurements of the shift in the water proton frequency. The choice of the water proton resonance, in order to carry out these measurements, is based on the fact that the relationship between the water proton resonance frequency and the temperature is, to a first approximation, independent of the composition of the biological tissue 410. The shift in the water proton frequency as a function of the temperature is, furthermore, linear and this linearity is not affected by the modifications of the biological tissue 410 induced by heat (Ishihara Y et al., Magn. Res. Med., 1995, 34, p. 814-823; Peters RD et al., Magn. Res. Med., 1998, 40, p. 454-459). The temperature dependence of the proton resonance frequency is 0.0094 ppm - K<sup>-1</sup> ("Fast magnetic-resonance temperature imaging"; by Zwart JA et al, 1996, J. Mag. Res. B, 112, p 86-90; "Fast lipid suppressed MR temperature mapping with echo-shifted gradient echo imaging and spectral-

*spatial excitation*", by Zwart JA et al., 1999, Magn. Res. Med., 42, p. 53-59).

The magnetic resonance signals coming from lipids  
 5 constitute a significant error source in the calculated  
 temperature maps, since the resonance frequencies of  
 the protons of the lipids do not depend on the  
 temperature. The magnetic resonance signals coming from  
 the lipids are therefore removed, by using selective  
 10 excitation of water, in the manner described in the  
 document "*Fast lipid suppressed MR temperature mapping  
 with echo-shifted gradient echo imaging and spectral-  
 spatial excitation*", by Zwart JA et al., 1999, Magn.  
 Res. Med., 42, p. 53-59.

15 The use of means for evaluating and numerically  
 processing the spatial temperature distribution 320 has  
 also already been described in the document "*Fast lipid  
 suppressed MR temperature mapping with echo-shifted  
 20 gradient echo imaging and spectral-spatial excitation*",  
 by Zwart JA et al., 1999, Magn. Res. Med., 42, p. 53-  
 59.

25 One implementational example of the method for  
 regulating the heat treatment equipment, according to  
 the present invention, is described in detail below.

30 Figure 4 shows schematically a flow diagram of  
 this particular mode of implementing the method  
 according to the invention.

According to this implementational example, the  
 method according to the invention comprises:  
 - a step 1 for estimating the heat diffusion  
 35 coefficient ( $\alpha_1$ ) and the absorption coefficient ( $\alpha_2$ ) of  
 the focused ultrasound in the biological tissue 410;  
 - a step 2 for defining by the user, a temporal change  
 profile of the desired temperature;



- a step 3 for acquiring an MRI image;
- a step 4 for calculating the spatial distribution of the phase at the focal point 460 and in its surroundings;
- 5 - a step 5 for establishing the spatial temperature distribution at the focal point 460 and its surroundings;
- a step 6 for evaluating the temperature gradients of the focal point 460 and in its surroundings;
- 10 - a step 7 for determining the new power having to be delivered by the generator 120; and
- a step 8 for changing the energy level applied by the generator 120.

15 Steps 3 to 8 are carried out in a loop, in order  
to reach and to follow the temporal change profile of  
the desired temperature, defined in step 2.

For a given element of the transducer 110, the electrical power  $P(t)$  transmitted to a sample is determined by the means for determining the power value 320. Its value may therefore be altered directly by the control unit 300. It is obtained on the basis of the equation:

$$P(t) = \frac{1}{\alpha_2(T_{\max})} \left[ \frac{d\Theta(t)}{dt} - \alpha_1(T_{\max}) \cdot \nabla^2 T_{\max}(t) + a \cdot [\Theta(t) - T_{\max}(t)] + \frac{a^2}{4} \cdot \Delta(t) \right]$$

This equation is established by considering the following.

The focal point is defined by  $\vec{r} = (0, 0, 0)$ . The temporal change and the maximum temperature of the focal point then corresponds to  $T_{max}(t) = T(0, 0, 0, t)$ . Let us denote the predetermined profile of the desired temporal change in the maximum temperature  $T_{max}(t)$  by  $\Theta(t)$ . As indicated above, this profile is defined

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before the start of each experiment. For example, this profile  $\Theta(t)$  comprises a rise of  $10^\circ\text{C}$  over 100 s. This rise step follows the change of a half period of the cosine function. It is followed by a period where the temperature is constant ( $10^\circ\text{C}$  above the starting value), for 250 s.

The maximum temperature  $T_{\max}(t) = T(0,0,0,t)$  may be controlled only at the focal point. This is because the geometry of the transducer and the spatial distribution of the refractive index in the biological tissue determines the acoustic field. Consequently, the temperature change other than in the focusing zone involves functions dependent on the space coordinate  $\vec{r}$  and on the temperature  $T$ . The acoustic power field  $\rho(\vec{r})$ , the heat diffusivity tensor  $\hat{\alpha}_1(\vec{r}, T)$  in the biological tissue and the absorption coefficient  $\alpha_2(\vec{r}, T)$  for the focused ultrasound are then related by the equation:

$$\frac{\partial T(\vec{r}, t)}{\partial t} = \vec{\nabla} \cdot [\hat{\alpha}_1(\vec{r}, T) \cdot \vec{\nabla} T(\vec{r}, t)] + \alpha_2(\vec{r}, T) \cdot \rho(\vec{r}) \cdot P(t). \quad [1a]$$

When the diffusivity is isotropic and varies slowly in space, equation 1a is simplified to give the equation 1b, where  $\alpha_1(\vec{r}, T) = \frac{1}{3} \text{Tr}[\hat{\alpha}_1(\vec{r}, T)]$  is a scalar field and  $\nabla^2$  is the Laplacian operator defined by  $\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}$ :

$$\frac{\partial T(\vec{r}, t)}{\partial t} = \alpha_1(\vec{r}, T) \cdot \nabla^2 T(\vec{r}, t) + \alpha_2(\vec{r}, T) \cdot \rho(\vec{r}) \cdot P(t) \quad [1b]$$

It should be noted that the functions  $\alpha_1(\vec{r}, T)$  and  $\alpha_2(\vec{r}, T)$  are not accurately known at the start of heating.

The acoustic power field of a spherical element of a focused ultrasound transducer corresponds approximately to a Gaussian distribution about the focal point 460, with an attenuation radius at 6dB denoted  $R_0$ . The specific diffusion time  $\tau$  is defined by  $\tau = \frac{R_0^2}{2\alpha_1}$ . For an ultrasonic wavelength of 1 mm, the order of magnitude of  $\tau$  is about 10 s.

10 The following objectives are established:

- 1) the temperature of the focal point 460 must reach the desired temperature as quickly as possible (that is to say in a time of the same order of magnitude as  $\tau$ ), this without oscillating or exceeding the desired value;
- 15 2) once this desired value is reached, the temperature must remain constant for a period predefined by the user.

20 The integral of the profile  $\Theta(t)$ , the integral of the time change of the maximum temperature of the  $T_{max}(t)$  observed experimentally and the difference between these two integrals, are respectively:

$$\Omega(t) = \int_0^t \Theta(t') dt' \quad [2a]$$

$$\Psi(t) = \int_0^t T_{max}(t') dt' \quad [2b]$$

$$\Delta(t) = \Omega(t) - \Psi(t) = \int_0^t [\Theta(t') - T_{max}(t')] dt' \quad [2c]$$

25

By using these expressions, equation 1b, giving the temperature change at the focal point, can be expressed as a function of  $\Delta(t)$ :

30

$$\frac{d^2 \Delta(t)}{dt^2} = \frac{d\Theta(t)}{dt} - \alpha_1(T_{\max}) \cdot \nabla^2 T_{\max}(t) - \alpha_2(T_{\max}) \cdot P(t) \quad [3]$$

where  $\vec{r} = (0,0,0)$  is omitted and  $\rho(0,0,0) = 1$ .

5 In equation 3, the parameter which it is desired to control directly is the power  $P(t)$  of the focused ultrasound. Let us note that a second order differential equation which is linear in  $\Delta(t)$ , can be advantageously used by the control unit 300, in a way  
10 similar to a PID control system. The reason for this is that the solution for  $\Delta(t)$  of such an equation tends asymptotically toward zero, and that this is the same for its first derivative. If the first derivative of  $\Delta(t)$  is equal to zero,  $T_{\max}(t)$  overlaps with the pre-  
15 determined profile of the temporal change in the temperature  $\Theta(t)$ . This constitutes the fundamental idea of the control method implemented by the control unit 300. Thus, we can rewrite equation 3 in the form of a linear differential equation of second order in  $\Delta(t)$  of  
20 the type:

$$\frac{d^2 \Delta}{dt^2} + a \cdot \frac{d\Delta}{dt} + \frac{a^2}{4} \cdot \Delta = 0 \quad [4]$$

25 So as to obtain the expression wanted for equation 4 from equation 3,  $P(t)$  is rewritten with the following expression:

$$P(t) = \frac{1}{\alpha_2(T_{\max})} \left[ \frac{d\Theta(t)}{dt} - \alpha_1(T_{\max}) \cdot \nabla^2 T_{\max}(t) + a \cdot [\Theta(t) - T_{\max}(t)] + \frac{a^2}{4} \cdot \Delta(t) \right] \quad [5]$$

30 Equation 5 corresponds to the central equation used to calculate directly the power level of the focused ultrasound. From the solution of the second order differential equation, corresponding to equation



ablation procedures) and the physiological parameters such as perfusion, are not accurately known.

Any error which may affect  $\alpha_1$  and  $\alpha_2$  may be treated  
 5 as a parameter error in a control loop, according to a linear model. Thus, estimates of the initial values of  $\alpha_1$  and  $\alpha_2$  will be chosen, then used during the heating procedure, in order to calculate the ultrasonic power, according to equation 5.

10

Theoretical analysis of the effect of the error on the parameters  $\alpha_1$  and  $\alpha_2$  in equation 5 highlights the following effects:

1) an incorrect estimate of the parameter  $\alpha_2$  decreases  
 15 the efficiency of the regulation loop; the possibility of exceeding the desired temperature or too small a value of the determined temperature may in fact ensue; this then has the consequence of increasing the convergence time. Whatever the case, even in these  
 20 conditions, the experimental temperature always tends asymptotically toward the predetermined profile of the temporal change in temperature.

2) an incorrect estimate of the parameter  $\alpha_1$  leads to a constant offset, in the zone where the profile  $\Theta(t)$  is  
 25 flat, of the temperature values, between the temperature values measured experimentally and the profile  $\Theta(t)$ . This offset is proportional to the first derivative, with respect to time, of the Laplacian, multiplied by the absolute error in  $\alpha_1$ , times  $a^{-2}$ . In  
 30 order to estimate this effect, the derivative of the Laplacian has been determined by using linear regression of the curve shown in figure 5, between 150 and 250 s. Its value is about  $0.01 \text{ K.mm}^{-2}.\text{s}^{-1}$ , which leads to a temperature offset of approximately  $0.1^\circ\text{C}$ .  
 35 Thus, the error in the actual temperature should not be directly observed because of the limitation in the accuracy of the thermometric measurements by magnetic resonance, because of the noise.

The influence of the error in the values  $\alpha_1$  and  $\alpha_2$  on the efficiency of the temperature control by the heat treatment equipment according to the invention is  
 5 studied experimentally, on a piece of fresh meat, below.

According to step 1, the parameters  $\alpha_1$  and  $\alpha_2$  must be estimated. This is carried out, firstly from a  
 10 preliminary experiment with a constantly focused ultrasonic power. The parameter  $\alpha_1$  is calculated from the derivative, with respect to time, of the temperature at the focal point, divided by the mean (over five MRI images) of the Laplacian. It is  
 15 calculated immediately after extinction of the focused ultrasound emission and is expressed in  $\text{mm}^2/\text{s}$ . The parameter  $\alpha_2$  (the rate at which energy is applied to the focal point taking into account the spatial power distribution of the focused ultrasound) is calculated  
 20 from the derivative, at the initial time, of the temperature of the focal point, with respect to the power of the focused ultrasound (when the focused ultrasound is emitted and the diffusion is negligible, see equation 1). It is expressed in  $\text{K.s}^{-1}(\text{mV})^{-2}$ . The  
 25 accuracy estimated for  $\alpha_1$  and  $\alpha_2$  is better than 10%, as may be deduced from repeated experiments. The numerical values obtained in this way may be directly used in equation 5 in order to calculate, each time a new temperature map is available, the actual value of the  
 30 power having to be supplied by the generator 120.

According to step 2, a profile of the desired temporal change in the temperature at the focal point is defined before the start of each experiment. This  
 35 profile comprises an increasing initial part, corresponding to a half period of the cosine function, followed by a constant temperature part. The first derivative of the curve corresponding to this profile





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means 350 for controlling the energy generating means 100.

5 The total calculation duration for processing each spatial temperature distribution map, that is to say each cycle of the set of steps 3 to 8 described hereinabove, is less than 250 ms.

10 To analyze the tolerance with respect to errors in the initial estimates of  $\alpha_1$  and  $\alpha_2$ , several heating procedures have been implemented with the parameters  $\alpha_1$  and  $\alpha_2$  varying over a large range. This range goes from 0 to 300% of a predetermined value, for  $\alpha_1$ , and from 40 to 250%, for  $\alpha_2$ . This predetermined value is that  
15 obtained from preliminary measurements outlined above. Waiting times of 30 minutes have been introduced between successive experiments with the aim of reaching the temperature base lines which are spatially uniform in the sample and identical for each experiment. Nine  
20 representative results are recorded in figure 6. These results show that the system has a large tolerance with respect to errors in the estimates of the initial values of  $\alpha_1$  and  $\alpha_2$ . Be that as it may, it is possible to note that the control loop becomes unstable only  
25 when  $\alpha_1$  is heavily overestimated. This instability is exacerbated when  $\alpha_1$  is heavily overestimated and  $\alpha_2$  is underestimated. This effect may be attributed to the experimental noise of the temperature measurements by MRI. When a calculation affected by the noise leads to  
30 an overestimate in the value of the Laplacian (the second derivatives being sensitive to the noise), the power of the focused ultrasound applied increases in a ratio equal to  $\alpha_1 \cdot \epsilon / \alpha_2$ , where  $\epsilon$  is the overestimate of the Laplacian. An increase in the power of the focused  
35 ultrasound leads to a strong increase in the Laplacian in the biological tissue and, as a result, a new increase in the power of the focused ultrasound. This positive reaction will cease after a time approximately

equal to  $2/a$ , by virtue of a negative reaction in the control loop. This explains the periodicity of the instability in this extreme case.

5        The strength of the negative reaction on the regulation loop corresponds to the parameter  $a$ . This is because, as we have seen above, the parameter  $a$  is equal to twice the inverse of the characteristic response time  $t_r(a=2/t_r)$ . The value of this parameter is  
10 indicated for each example of figure 6. In general, the values of  $a$  of  $0.1 \text{ s}^{-1}$  to  $0.2 \text{ s}^{-1}$  are enough to reach temperature rise times similar to those of the profile  $\Theta(t)$ , even when extremely incorrect values of  $\alpha_1$  and  $\alpha_2$  are used. It is only in the extreme case where  $\alpha_1$  is  
15 heavily underestimated and  $\alpha_2$  is overestimated (see figure 6, at the bottom right), that  $a$  must be increased up to the value of  $0.40 \text{ s}^{-1}$  in order to obtain an overlap with the predetermined profile of the temporal change of the temperature. The optimum value  
20 of  $a$ , found experimentally (in all cases, except the cases with extreme errors in  $\alpha_1$  and  $\alpha_2$ ), is  $0.2 \text{ s}^{-1}$ , the response time of the corresponding regulation loop being 10 s. When the strength of the negative reaction is increased, a faster correction of the errors in the  
25 initial parameters is obtained, but the amplitude of the power of the focused ultrasound and of the temperature fluctuations around the predetermined value is also increased.

30        The implementation and the performance of the treatment equipment according to the invention described above are illustrated below by means of two examples.

35        Example 1: Use of the heat treatment equipment, according to the present invention, in the context of *in vitro* measurements

According to this example, a temperature rise protocol of 10°C is implemented on a sample of fresh meat. The initial temperature is equal to 15°C. With this protocol, no irreversible alteration of the biological tissue 410, resulting from this temporal change profile of the temperature, is expected. Figure 7 shows the change in the maximum temperature as a function of time. In the flat part of this curve, the mean temperature rise is 9.97°C, with a standard deviation of 0.19°C. This standard deviation must be compared with that, equal to 0.18°C, which is obtained by the temperature measurements carried out without heating by focused ultrasound (that is to say that which corresponds to the base line of noise in the temperature measurements). Figure 5 shows the directly calculated Laplacian. The attenuation observed over the constant temperature part corresponds to the decrease in temperature gradients around the focal point. As for the amplitude of the directly applied power, this is shown in figure 8. Because of the measurement noise, the calculated value of the Laplacian and the amplitude of the power supplied by the generator have a fluctuation of about 10%, approximately. This has only a small effect on the resulting temperature, since the fluctuation frequency (that is to say the inverse of the temporal resolution of the map by magnetic resonance) is much greater than the inverse of the response time ( $\tau$ ) specific to the heating of the biological tissue 410.

30

Figure 9 shows the temperature stability obtained with a profile having three stages (15, 25, 30°C). The standard deviation is 0.35°C, 0.36°C and 0.40°C, respectively, for temperature rises to 15°C, 25°C and 30°C. Results shown in figure 9 confirm the high temperature stability over a large range of temperature increase, of the system for regulating the heat treatment equipment according to the present invention.

35

Example 2: Use of the heat treatment equipment, according to the present invention, in the context of *in vivo* measurements

5

By adopting a procedure similar to that implemented in the case of example 1, experiments were carried out *in vivo*, on a rat's thigh. The corresponding results are shown in figure 10. The  
10 temporal resolution is 0.5 s. The mean temperature, between 90 and 120 s after the start of the experiment, is 54.9°C (the value of the profile to be reached is 55°C) with a standard deviation of 0.33°C. Figures 7, 9 and 10 show that it is possible to control the  
15 temperature with an accuracy close to that given by the temperature measurements carried out *in vitro* or *in vivo*.

An embodiment of the invention corresponding to  
20 local hyperthermia treatment equipment by focused ultrasound, controlled by MRI, has been described above, but the invention covers a much wider range of heat treatment equipment. Also, it will be understood that the invention may be generalized to the cases  
25 where the heat is, for example, provided by a laser, microwaves or radiofrequency waves, focused ultrasound, etc. It will also be understood that other means of measuring the temperature may be used in the heat treatment equipment according to the present invention,  
30 in the place of MRI.

Similarly, the evaluation and numerical processing of the spatial temperature distribution have been described above as being carried out using the  
35 Laplacian. Other means of carrying out this evaluation may be used without departing from the scope of the invention.

# CLAIMS

1. Equipment for the heat treatment of a target zone of biological tissue (410), comprising:
  - 5 - energy generating means (100) for supplying energy locally in the target zone;
  - means (200) for measuring and recording the temperature in the target zone;
  - a control unit (300) comprising means (330) for  
10 determining, from the temperature measured in the target zone, the amount of energy having to be supplied to the target zone, and means for controlling (350) the energy generating means (100) to deliver this power value;
  - 15 characterized in that the control unit (300) furthermore comprises means (320) of numerically processing, point by point, the spatial temperature distribution in the target zone and its surroundings, in order to calculate temperature gradients.
  - 20
2. The heat treatment equipment as claimed in claim 1, characterized in that the control unit (300) furthermore comprises means (340) for estimating the  
25 local heat energy losses, from an estimate of the heat conduction and of the spatial temperature distribution in the target zone and its surroundings.
3. The heat treatment equipment as claimed in one of the preceding claims, characterized in that the energy  
30 generating means (100) emit focused ultrasound.
4. The heat treatment equipment as claimed in one of the preceding claims, characterized in that the means (200) for measuring and recording the spatial  
35 temperature distribution comprise a magnetic resonance imaging apparatus.

- 25 -

5. The heat treatment equipment as claimed in one of the preceding claims, characterized in that it comprises means for evaluating the spatial distribution, in the target zone and its surroundings, of the energy supplied to the target zone.

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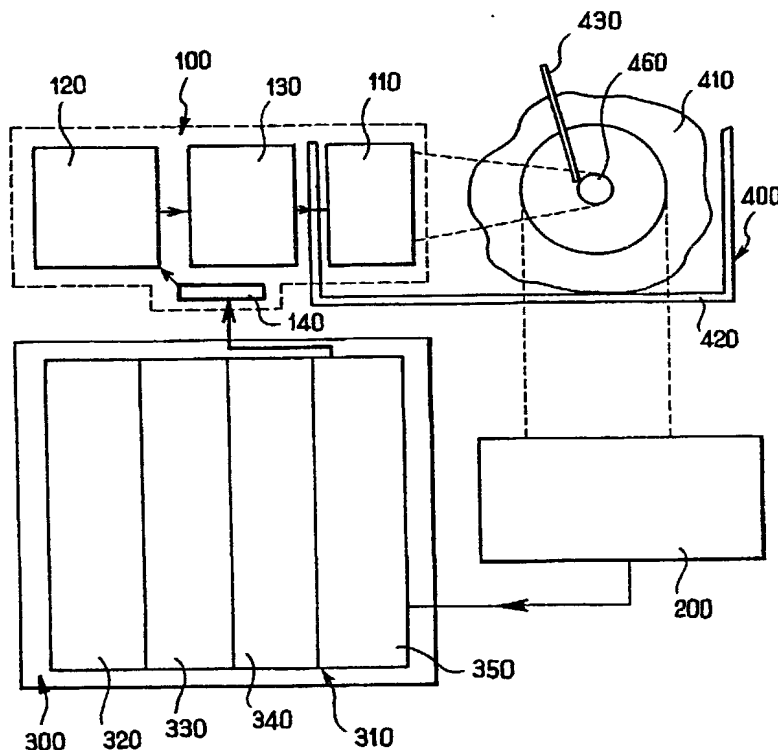
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[Suite sur la page suivante]

(54) Title: SET FOR HEAT TREATMENT OF BIOLOGICAL TISSUES AND METHOD USING SAME

(54) Titre: ENSEMBLE DE TRAITEMENT THERMIQUE DE TISSUS BIOLOGIQUES ET PROCEDE DE MISE EN OEUVRE  
DE CET ENSEMBLE



(57) Abstract: The invention concerns a set for heat treatment of a target zone of a biological tissue (410), comprising energy-generating means (100) to supply energy locally in the target zone, means (200) for measuring and recording temperature in the target zone, a control unit (300) comprising means (330) for determining, on the basis of temperature measured in the target zone, the amount of energy required to be supplied to the target zone, and means for controlling (350) the energy-generating means (100) to deliver said power value. The invention is characterised in that the control unit (300) further includes means for point by point digital processing (320) of the spatial distribution of temperature in the target zone and its immediate surroundings.

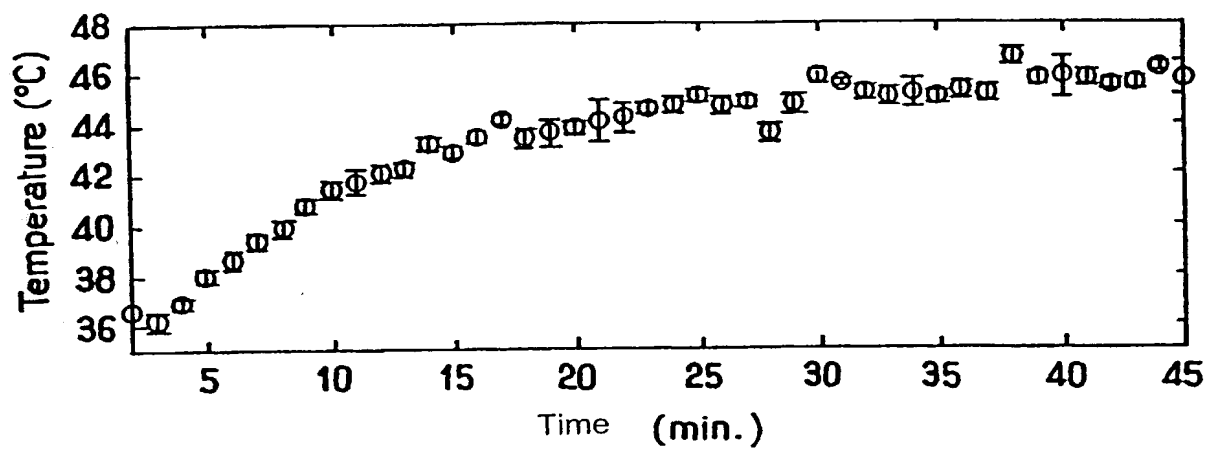
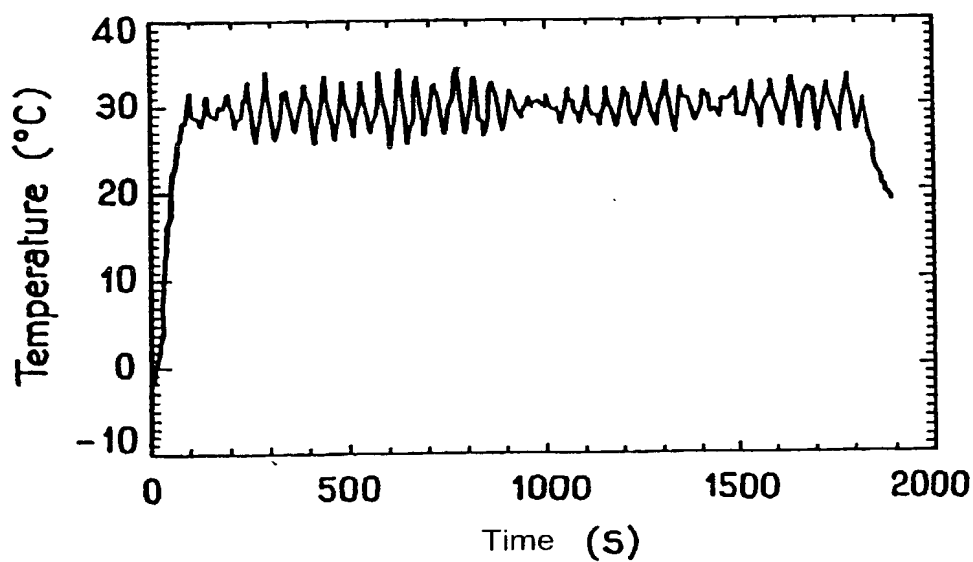
(57) Abrégé: La présente invention concerne un ensemble pour le traitement thermique d'une zone cible d'un tissu biologique (410), comprenant des moyens générateurs d'énergie (100) pour fournir localement de l'énergie dans la zone cible; des moyens (200) pour mesurer et enregistrer la température dans la zone cible; une

unité de

[Suite sur la page suivante]

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FIG. 1PRIOR ARTFIG. 2PRIOR ART



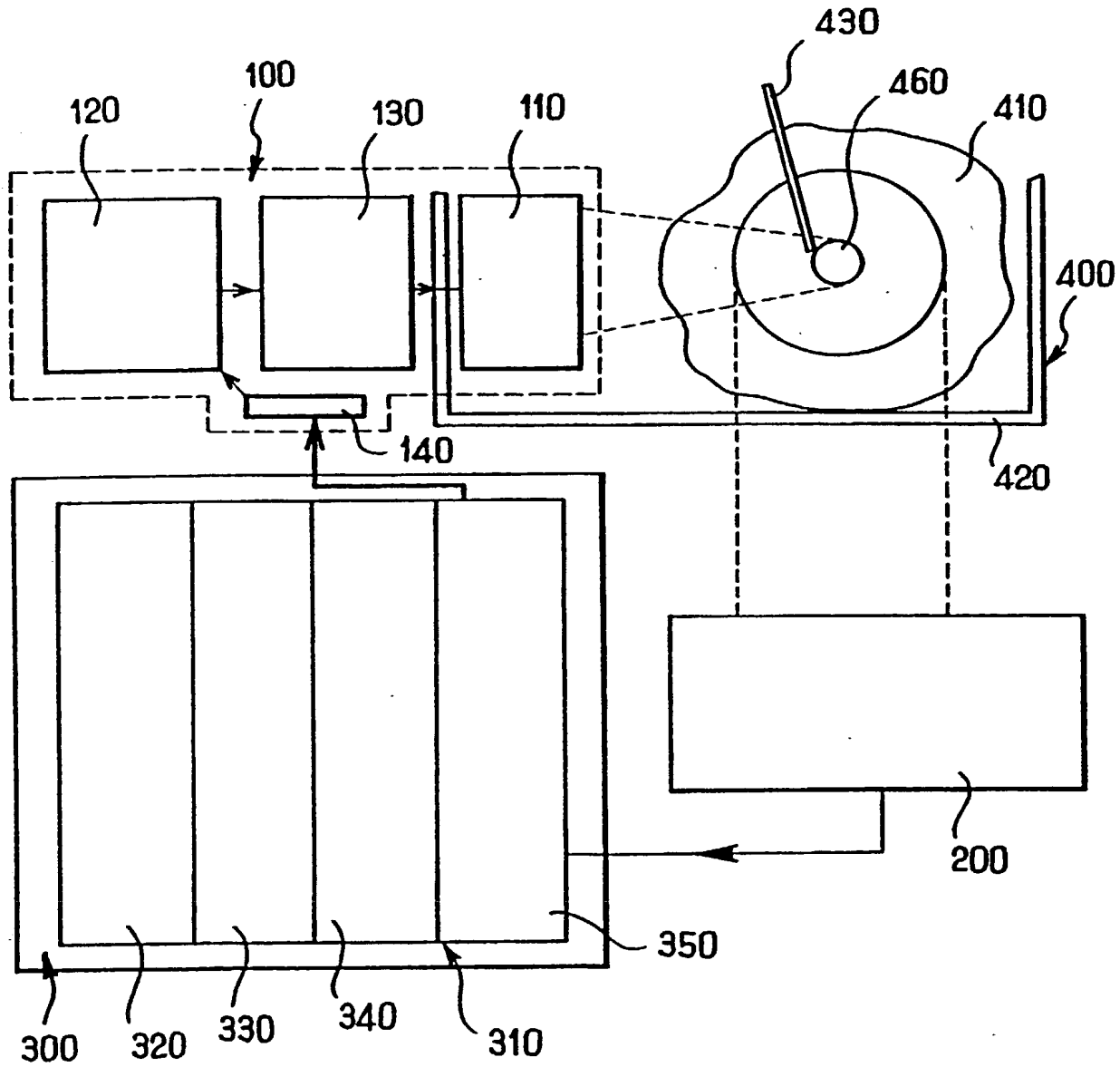
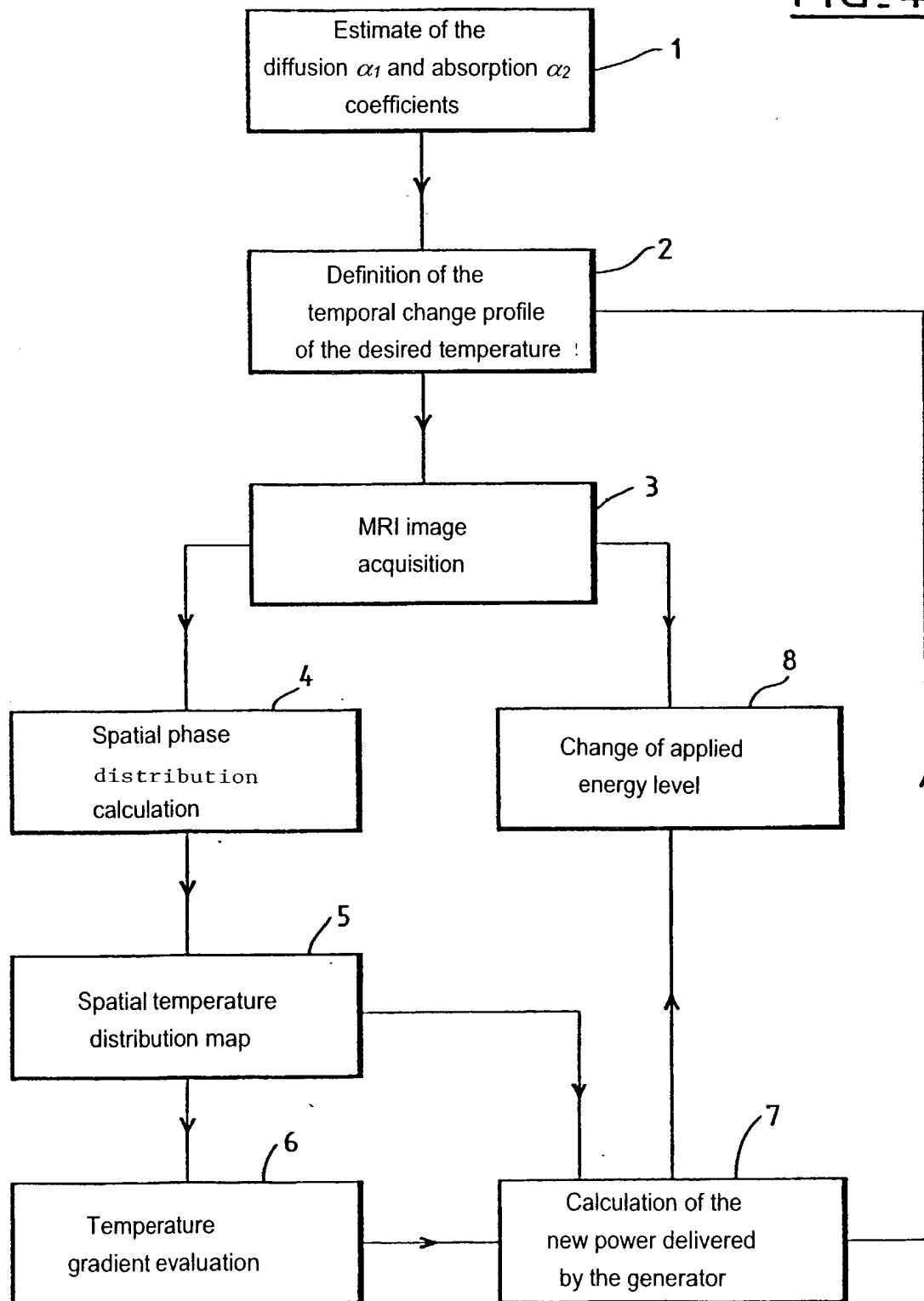
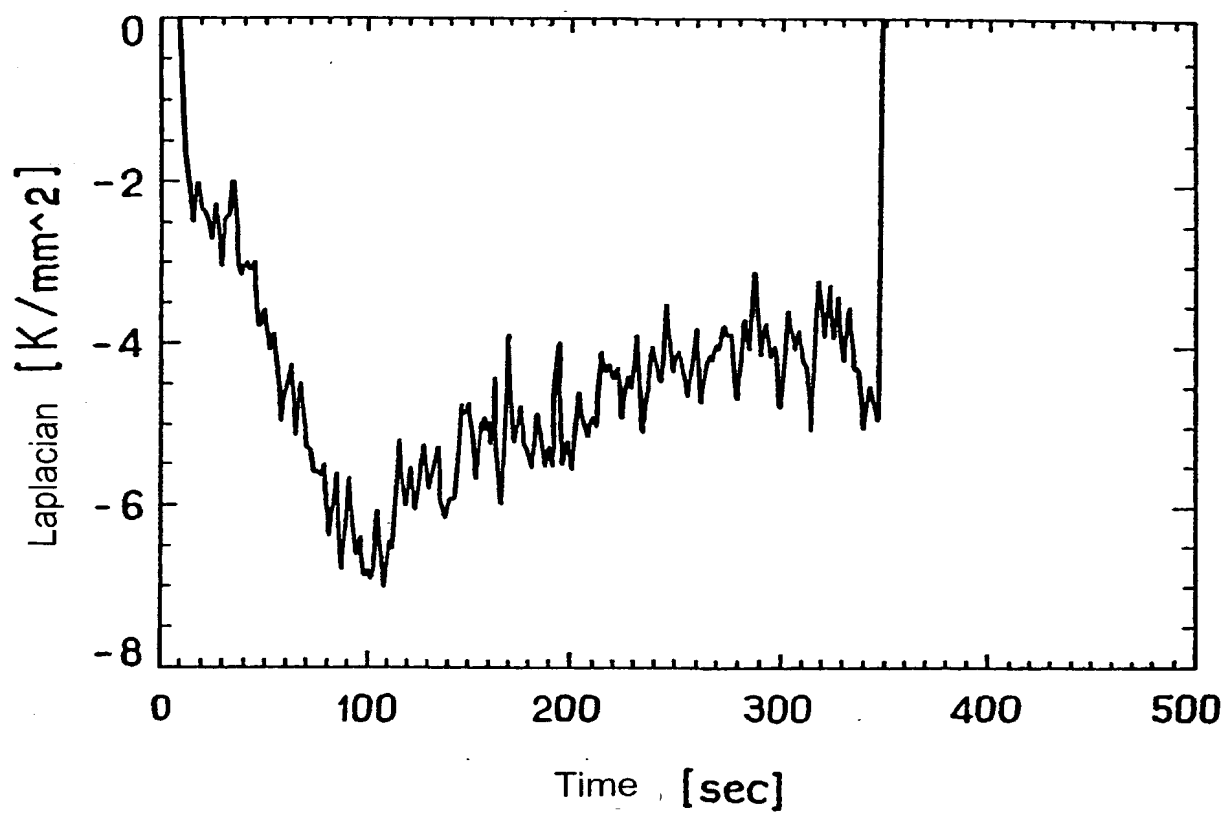


FIG. 3

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FIG. 4

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FIG.5

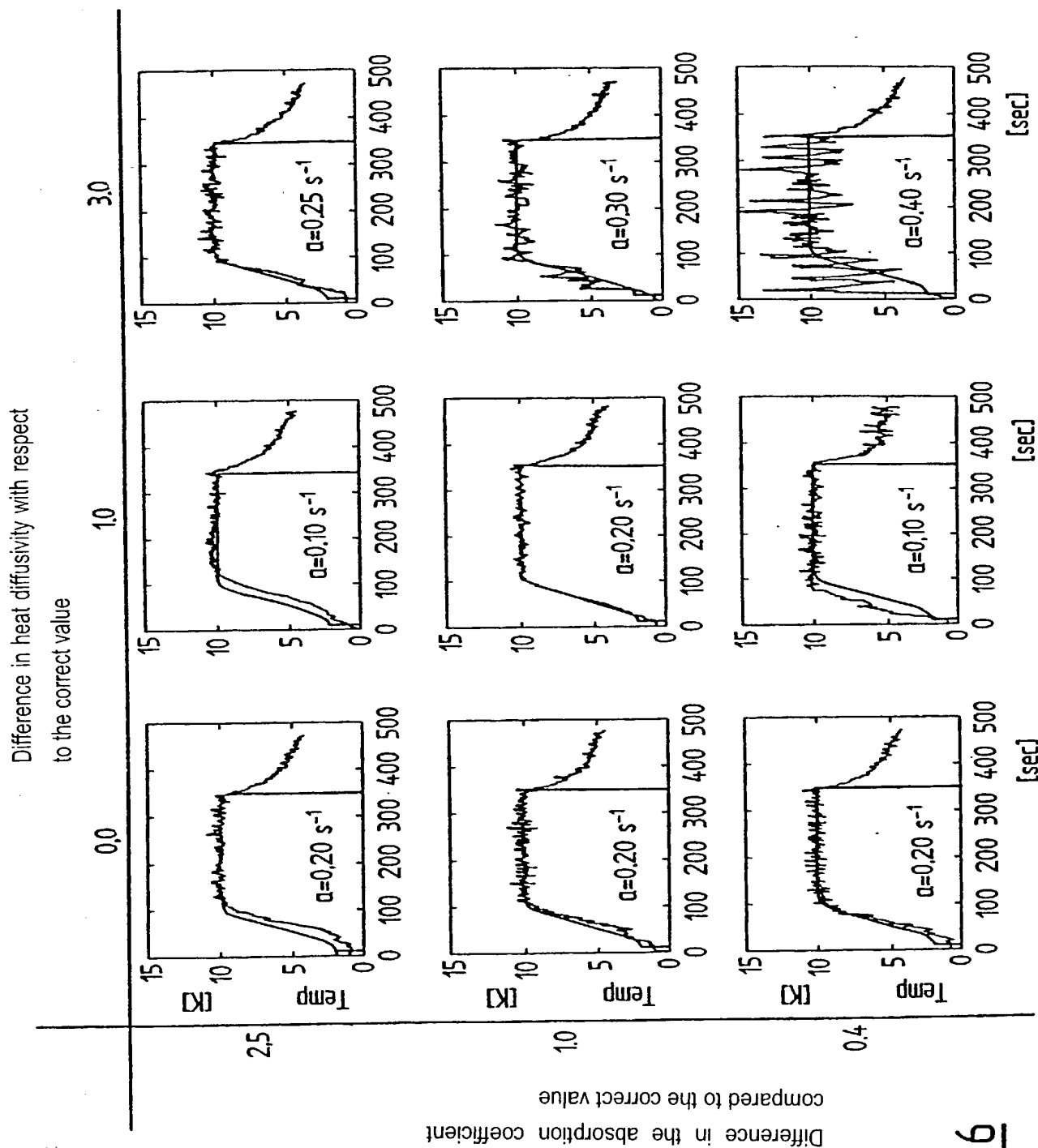


FIG. 6

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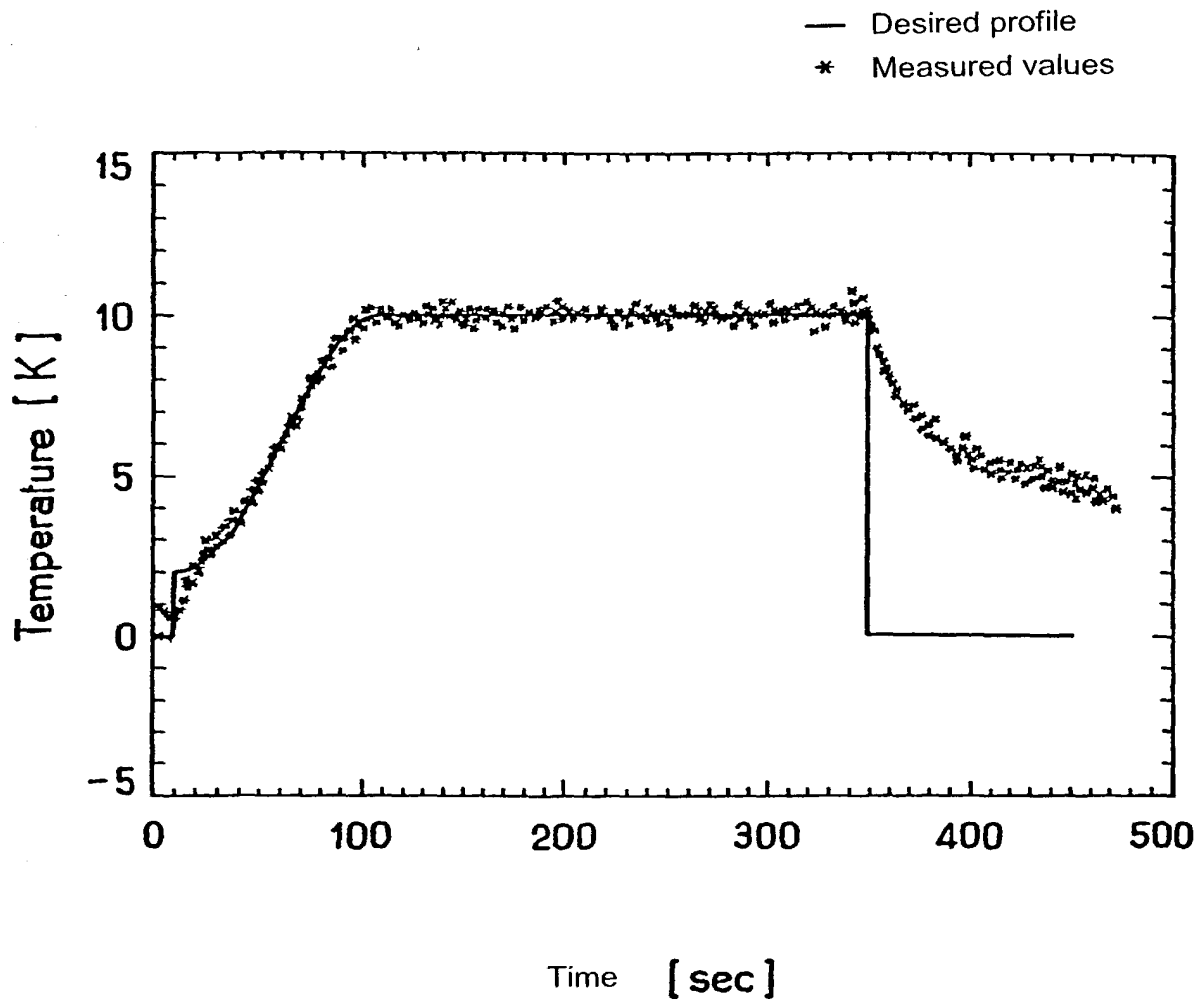
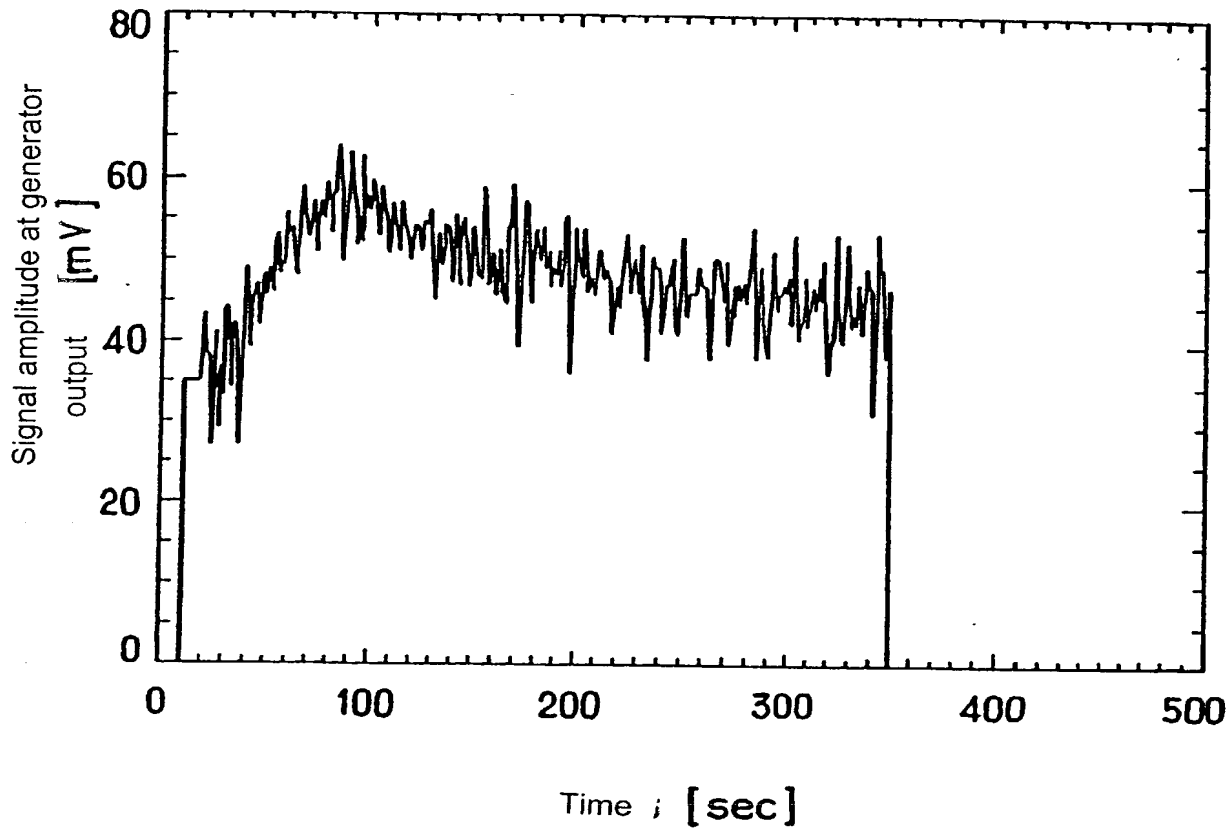
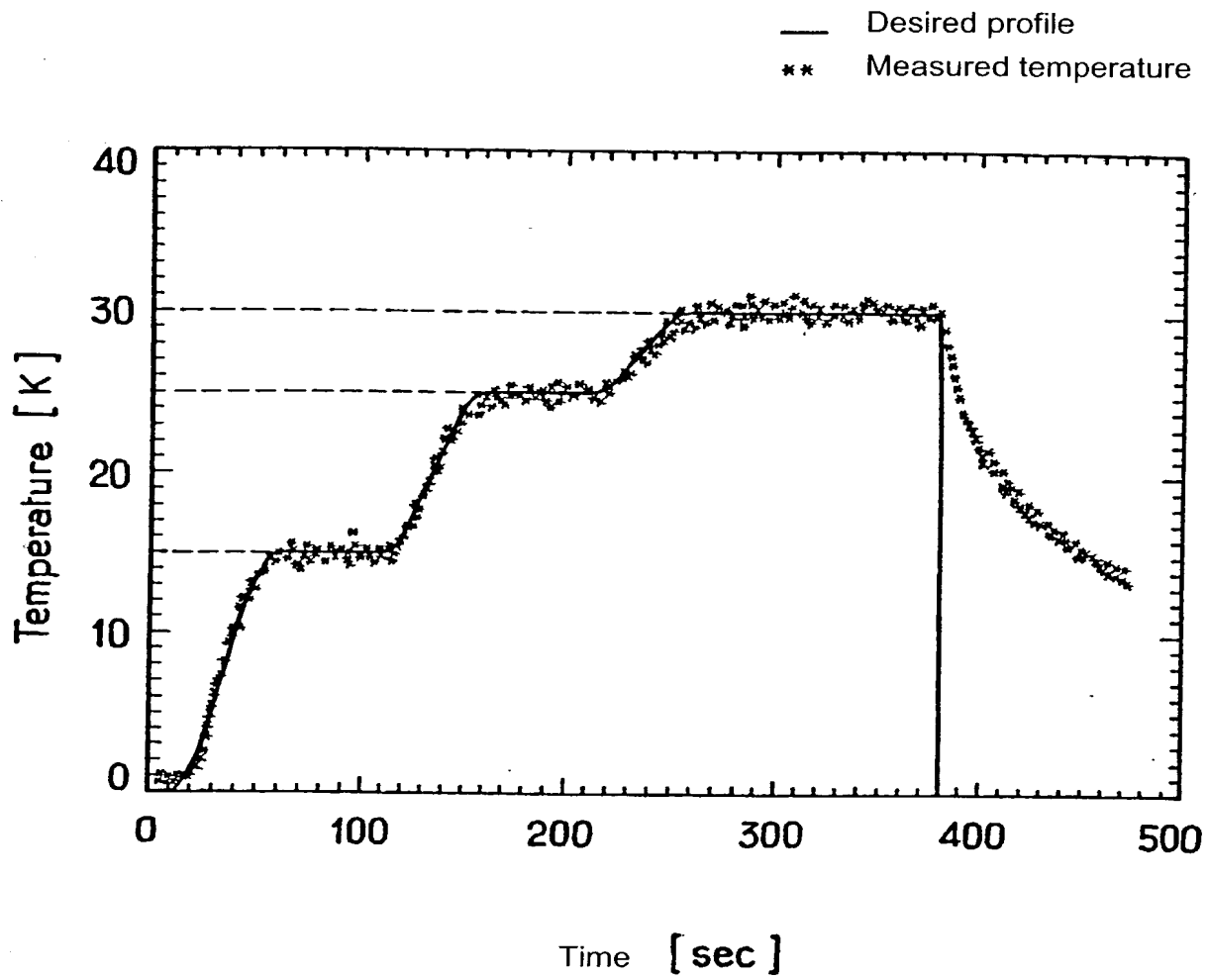


FIG.7

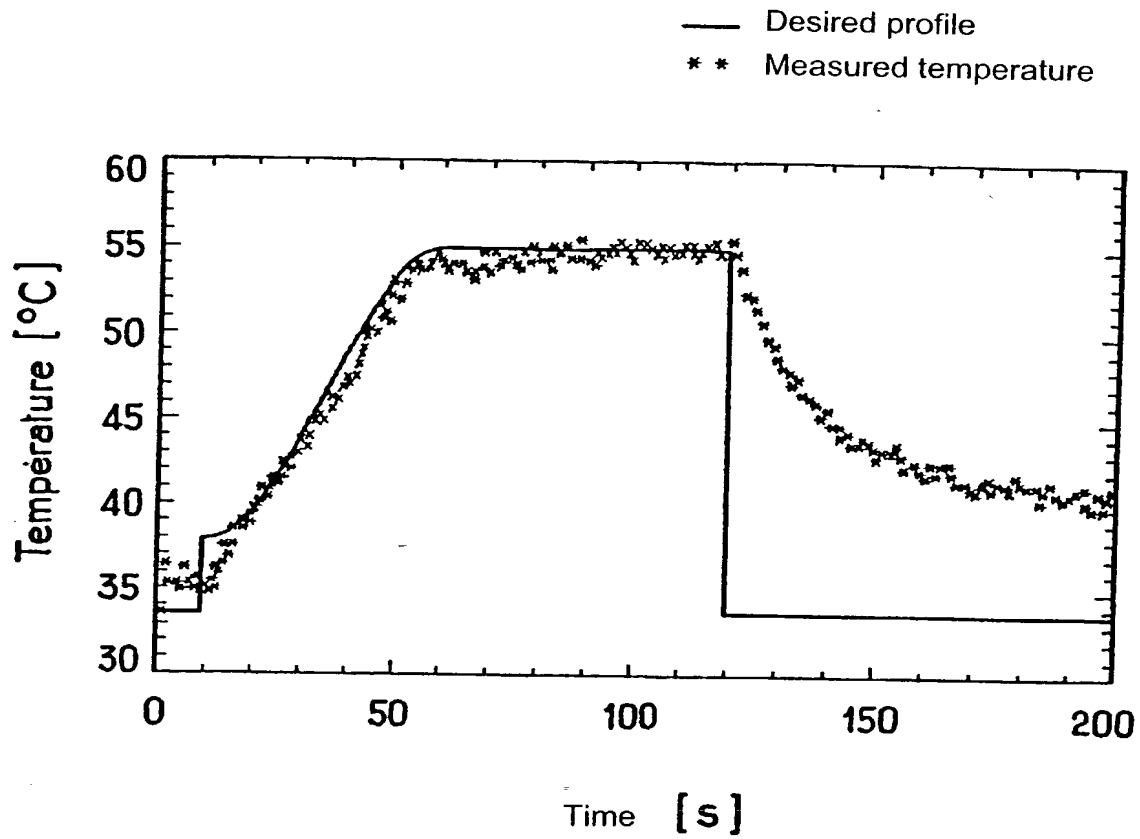
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FIG. 8

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FIG\_9

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FIG\_10





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**DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below, next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

SET FOR HEAT TREATMENT OF BIOLOGICAL TISSUES AND METHOD USING SAME

the specification of which

is attached hereto  
was filed on September 12, 2000 as  
Application Serial No. PCT/FR00/02506  
And was amended on  
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I do not know and do not believe that the same was ever known or used in the United States of America before my invention thereof, or patented or described in any printed publication in any country before my invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, and that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to this application.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Section 199, of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor(s) certificate having a filing date before that of the application on which priority is claimed:

**Prior Foreign Application(s)****Priority Claimed**

<b>99 11418</b> (Number)	<b>France</b> (Country)	<b>13.09.1999</b> (Day/Month/Year Filed)	<b>XX</b> Yes	No
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

**PCT/FR00/02506**

(Application Serial No.)

**12.09.2000**

(Filing Date)

**Pending**

(Status - patented, pending, abandoned)

(Application Serial No.)

(Filing Date)


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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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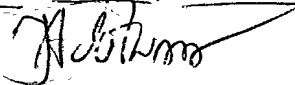
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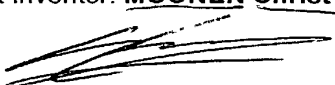
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